Low- & mid-density **genotyping services**

CGIAR Excellence in Breeding Platform

World-class services at reduced cost for CGIAR and NARS breeding programs

Low-cost, accurate genotyping services offer several different applications with the potential to accelerate the production of improved varieties and create efficiencies in crop breeding programs.

However, smaller breeding programs may not have the know-how or sufficient demand for genotyping to be beneficial and economical.

The CGIAR Excellence in Breeding Platform (EiB) aggregates demand from several breeding programs to negotiate competitive genotyping prices with vendors, offering a streamlined service along with application-oriented training and advice.

This document introduces the EiB low-density and mid-density genotyping services for potential users.

Genotyping / sequencing tools & services

Contact:
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How does it work?

The low- and mid-density services are based on contracts negotiated by EiB with validated DNA extraction and genotyping service vendors based on anticipated bulk demand across eligible CGIAR and national agricultural research (NARS) institutions.

The process of sending samples and receiving high-quality data is streamlined and integrated with the support and advice provided by EiB. Available worldwide, the process also requires minimal or no phytosanitary paperwork.

1. Contact EiB for advice
2. Fill out order form
3. Sampling and documentation
4. Shipping
5. Genotyping
6. Data return

How can I get started?

Start by getting in touch with EiB genotyping contacts for advice, procedures and costing/ budget questions. For example, it is important to follow the correct material sampling protocols to obtain high-quality genotyping data.

EiB can also provide advice on related areas such as the optimum use of genotyping in breeding schemes or biometrics and data management.

The main points to consider when planning to genotype are:

- The purpose of genotyping.
- The organism being genotyped.
- Number of samples and technical repetition.
- Experience with primer design (this service can be provided by vendors).
- Available budget.

Mid-density genotyping

Methods: DArTag

Density: Over 300 markers

Time: 15 business days upon receipt by lab.

Cost: US $10-11 per sample

Key features

DArTag is a targeted genotyping method run on selected SNPs (or INDELS), whether on markers discovered by DArTseq or elsewhere.

The service is primarily intended for genomic selection applications, but other uses are possible if the genetic material is not significantly different from the germplasm pool for which DArTag assays were developed.

Mid density panels can also be used for diversity studies, material fingerprinting or background recovery in marker assisted selection (MAS) to complement low-density genotyping. Quality control is also possible, though low-density genotyping is more cost-effective.

DArTag has been successfully applied to a number of polyploid crops, including in an initial pilot EiB project on tetraploid potato. Service providers together with users will determine the optimal report format for polyploids.

Data

DArT returns multiple datasheets, showing marker calls and marker “counts” for each allele and sample in a binary format. Raw data (fastq) is available at the point of ordering, at a cost of $200 per service; other formats may be possible upon request.

How does DArTag work?

DArTag uses a single oligonucleotide to capture SNPs or indels, the target regions are then amplified and attached to a sample-specific barcode for sequencing and processing.

Compared to other techniques, DArTag can be used in assays up to 4,000 markers per panel under the EiB service agreement, in a simpler and cost-effective manner.

Sequencing depth is usually between 100-300 X, but depends on factors such as species genome size, genetic diversity (including heterozygosity frequency) and ploidy level, in addition to budget.

A **reference genome** - material/sample with known sequence for that specific marker - is used for all markers to avoid internal biases and errors in NGS data.

A **negative control** (water in place of DNA) is analyzed to detect contamination during the process.

A **positive control** is a high quality sample of a known and stable sequence for a trait of interest, used to detect potential technical issues.

Panel design

Detail requirements for assay design are provided in the SNP Information Panel Setup on the DArTag Order Form (link).

The Diversity Arrays Techology (DArT) platform has been successful in developing DArTag panels from data generated by several genotyping by sequencing methods.

While most of the DArTag markers of this type were developed from DArTseq markers, RADseq and some “GBS” markers were also successfully converted, including some “GBS” markers for common bean pilot project of EiB.

To use the available panels provided by DarT, new users must first consult with EiB to ensure the service agreement terms are met, and the owner of the panel is notified.

In the case that the CGIAR panels do not fit the organism, it is possible to use an existing DArT alternative upon discussion and agreement with vendors.

Regions with high divergence may reduce the specificity of the DArT oligonucleotide; measures to resolve issues identified in panel testing are discussed and agreed between partners and service provider.

Mid-density DArTag panels may be customizable into subsets to ensure maximum utility. Each assay order is sufficient for up to 100,000 runs.

Setting up new marker panels

Step 1: Contact service provider and EiB genotyping coordinator

Step 2: Submit marker sequences for assay design at Diversity Arrays Platform

Step 3: Assay order

Step 4: Provide verification samples (usually 384, 4 x 96) and minimum of one technical reference

Step 5: Finalize mid density panel for routine use

Costs and time estimates

The standard cost to set up a new marker is $12 per assay, with a negotiable discount above 2,000 assays (which can be aggregated across multiple crops and users). The standard lead time for new panel setup and assay synthesis is between 4-6 weeks.

Only samples that meet quality standards will be genotyped; users will be notified by email if this is the case, having the option to cancel or resubmit new samples (at a gDNA extraction and consumable cost of $2 per sample), or proceed with potentially longer turnaround time and lower than 95% data return.

Low-density genotyping

Methods: Kompetitive allele specific PCR (KASP)

Density: Recommended for up to 100 markers

Time: 10 business days upon receipt by lab.

Cost: US $2-6 per sample on average

Key features

Low-KASP genotyping is highly specific to a target: it can be used for QC analysis, drop-down selection, marker assisted selection as well forward breeding. Here, the markers are known, validated and in use.

Compared to other methods, KASP is cost-effective, has a short turnaround time and has a low error rate.

What is the optimal marker density?

The low-density platform recommended for applications up to 100 SNP markers: the optimal marker density depends on how good the marker is in predicting the phenotype.

Where can KASP markers be found?

A collection of KASP markers can be found on the EiB website: excellenceinbreeding.org/module3.

These markers have been validated by diverse CGIAR, NARS and advanced research institutes. If the background of the population is different from where the KASP markers were designed, users should validate these KASP markers in their own material.

Our markers are available in the public domain and so are free to use, though attribution is always advisable.

Troubleshooting problems

If unexpected genotyping results are received, the following issues could be considered:

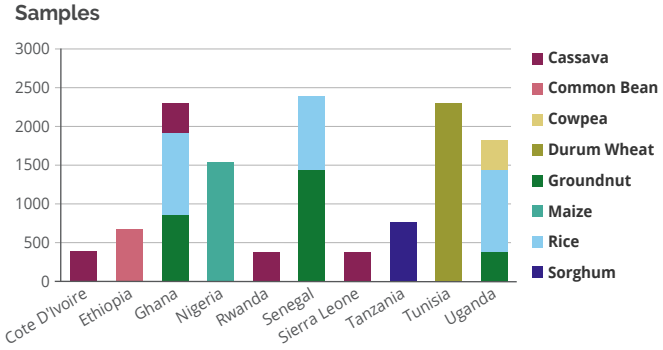
- The existence of different haplotypes.
- Origin of the SNP marker used (i.e. studies or literature).
- Ploidy of the working material (polyploid genetics are more complex).
- Primer design
- Technical issues.

These issues and other solutions are explained in accompanying training materials.

Our experience

Low-density genotyping services have been provided since 2017 under the High Throughput Genotyping Project (HTPG), covering most CGIAR mandate crops.

Demand for this service created more than $2 million in business value by 2019, meaning that the service is self-sustaining.



From September 2019 to December 2019, over 13,000 samples were genotyped for 10 crops in eight African countries.

Excellence in Breeding PLATFORM

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